GENETIC ANALYSIS OF BEAR CREEK/COTTAGE LAKE CREEK NATURALLY SPAWNING FALL-RUN CHINOOK

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Introduction

This report describes the genetic analysis of fall-run chinook salmon that were sampled on their spawning grounds in Bear Creek and Cottage Lake Creek during 1998. There were no previous genetic data on naturally spawning chinook in this Sammamish tributary system. The population is relatively small and its origins obscure. Managers were interested in determining its relationship to nearby hatchery and wild chinook populations, especially whether we could distinguish it as a discrete population or heavily influenced by strays from Issaquah Hatchery. Genetic data were already available for many hatchery and wild populations throughout Puget Sound, including Issaquah.

Our genetic characterization is done using allozyme gene loci. Allozymes are various forms of common metabolic enzymes whose genetically-encoded protein structure can be assayed by electrophoresis. Allozyme gene or allele frequencies have been collected for chinook salmon from Alaska to California during the last 20 years in order to create a coast-wide database for a variety of management purposes. This database was used in the recent National Marine Fisheries Service (NMFS) status review of chinook salmon to delineate Evolutionarily Significant Units (ESU) in response to ESA petitions. Data collected for Bear /Cottage Lake Creeks chinook complied with coast-wide standards. For those not familiar with genetics terminology, a glossary follows the end of this report.

Methods

Tissue samples of muscle, eye, heart, and liver were taken from adult chinook post-spawning. Field samplers collected tissues from 12 chinook in Bear Creek and 59 chinook in Cottage Lake Creek, for a total sample size of 71. Samples were kept on dry-ice and later stored at -75^{o} C. Scale samples were also taken and used for aging.

We used starch-gel electrophoretic methods to analyze genetic variation at 57 allozyme loci. The protocols employed were the same as used with any baseline population sample, and allowed us to screen for all known variant alleles in chinook salmon. To ensure accuracy of interpretation and data entry, allozyme phenotypes were often resolved in two or more tissues, and/or with two different gel buffers. We used a WDFW computer program to check for discrepancies from all sources of phenotype scores. The final product of laboratory analysis was a multi-locus genotype for each

chinook sampled.

Observed genotype frequencies at variable loci were compared to those expected in a population by Hardy-Weinberg (HW) equilibrium tests. Significant deviations from expected genotype ratios can be an indication of population mixture. Allele frequencies at all loci in the total sample were calculated from genotype data. Allele frequencies provided the characterization of the population that was used for comparisons with other discrete populations.

The other Puget Sound population samples used for comparative analyses are described in Table 1. I compared allele frequencies from these populations with those found in Bear/Cottage Lake Creeks sample using *G*-tests (log-likelihood ratio tests) to see if significant differences were detectable. One can expect allele frequencies to be divergent between two populations that are not exchanging genes at a high rate, and/or have different ancestries. A statistic called genetic distance was computed between all possible pairs of Puget Sound population samples based on allele frequencies at 42 loci, which are typically variable throughout the range of chinook salmon. Inter-relationships of populations based on these genetic distances were visualized by cluster analysis and three-dimensional scaling techniques.

Results

We found genetic variation at 22 loci in the Bear/Cottage Lake sample. This is slightly fewer variable loci than found in most Puget Sound chinook populations, which have on average 28 variable loci. One locus showed a significant ($p<0.05$) deviation from expected HW equilibrium due to a deficit of heterozygous genotypes. This was the only significant result among all loci tests, and was no more than that expected due to chance (5%).

I found significant differences ($p<0.05$) between allele frequencies of the Bear/Cottage Lake sample and those of all 21 population samples tested. All *G*-tests but one were actually significant at $p<0.01$. This lower probability level indicates a higher likelihood that samples came from separate breeding populations. Allele frequencies between Bear/Cottage Lake and Issaquah Hatchery samples were significantly different at $0.01<p<0.05$. Bear/Cottage Lake chinook had particularly distinctive allele frequencies at the sSOD-1 locus. They had a relatively low b allele frequency coupled with the highest c allele frequency among all Puget Sound samples.

The cluster analysis of genetic distances (Figure 1) among samples showed that Bear/Cottage Lake Creeks chinook were more closely aligned with South Puget Sound populations than with those from the Snohomish Basin and northwards. Among all pair-wise genetic distances calculated between Bear/Cottage Lake and other Puget Sound samples, the smallest distance was found with Issaquah Hatchery. However, genetic distances were smaller between Issaquah and several other samples, such as Green River and Puyallup hatcheries, than with Bear/Cottage Lake. Due to close relationships among South Sound hatcheries, the Bear/ Cottage Lake chinook sample was an outlier to the cluster containing Issaquah Hatchery.

An additional view of genetic distance relationships is shown using three-dimensional scaling techniques

in Figure 2. To allow more detail, I used only samples from 15 southern Puget Sound populations. Again, the Bear/Cottage Lake chinook sample was an outlier to a central cluster of South Sound hatchery samples.

Conclusions

The genetic characteristics found in the 1998 Bear/Cottage Lake Creeks spawner sample imply that the population is a discrete, self-sustaining unit. Although it showed least differentiation from the Issaquah Hatchery population, any gene flow from interbreeding with hatchery strays did not appear to have been large enough to cause homogenization of allele frequencies. It would be useful to know at what level, if any, wild spawners have been included in Issaquah Hatchery broodstocks. The lower than average number of variable loci and distinctive frequencies at sSOD-1 may indicate a large decline in population size, or that the population was originally founded by small numbers of spawners and has remained relatively small.

Managers were also interested in how Bear/Cottage Lake Creeks chinook compared genetically to wild chinook in the Cedar River, and they were clearly distinct from them. In the state-wide picture of chinook population genetics, Bear/Cottage Lake Creeks chinook are most closely related to South Puget Sound summer and fall-run hatchery and wild populations, versus any other group. Given the current data, it seems appropriate to treat the Bear/Cottage Lake Creeks population as an independent member of this Puget Sound group. I recommend another year of sampling to both increase sample size and test for temporal differences. Small populations often show annual variation in allele frequencies due to random events, a process known as genetic drift. In this case, multiple year samples often provide a better genetic characterization. The possibility exists that with additional data, interpretations and conclusions may be altered.

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GLOSSARY

allele - a particular form of a protein or DNA sequence (gene) at a locus

allozymes - enzymes produced by allelic forms of genes that may be differentiated by net charge, and detectable by electrophoresis and biochemical staining

electrophoresis - the separation of large molecules (such as proteins) in an electric field, using a medium such as a starch gel as a physical structure for the process

enzyme - proteins that catalyze metabolic reactions, acting with high specificity on target molecules

gene - a DNA sequence that encodes a product, such as a protein, and functions as the hereditary unit

GLOSSARY -continued

genotype - genetic attributes of an individual for a particular gene or locus, which are typically stated for the two alleles of paired nuclear genes

Hardy-Weinberg equilibrium - the expected genotypic frequencies in a randomly-breeding population based on a given set of allele frequencies at a locus

heterozygote - a genotype composed of two different alleles or genes, alternative to a homozygote in which both genes are the same

locus - the site of a gene (DNA sequence) within the chromosomes, or sometimes, the gene and its alleles

phenotype - the physical properties or manifestation of a genotype; for allozymes, banding patterns on the gel provide a representation of the genotype

Table 1. Other Puget Sound chinook population samples (n=21) used for comparative analyses with the 1998 Bear Creek/Cottage Lake Creek sample (n=71).

Name	Yrs. of collection	Number sampled
Skokomish River fall-run	98	121
Green River Hatchery fall-run	98	100
N.F. Stillaguamish River summer-run	87-88	106
S.F. Stillaguamish River fall-run	92-96	113
Suiattle River spring-run	85-90	548
Upper Sauk River spring/summer-run	86+94	147
Skagit Hatchery spring-run	90,93-94	254
Upper Skagit River summer-run	86+94	172
Skykomish River summer-run	89,93,96	178
Sultan River fall-run	87-89	95
Snoqualmie River fall-run	88	101
Green River Hatchery fall-run	87-88	200
Cedar River fall-run	93-94	107
Skykomish Hatchery fall-run	87	106
Puyallup Hatchery fall-run	92-93	150
Deschutes Hatchery fall-run	82,87	249
Hoodsport Hatchery fall-run	82,88	248
White River spring-run	92-93	400
South Prairie Creek fall-run	92-93	86
Newaukum Creek fall-run	92-93	144
Issaquah Hatchery fall-run	92	99

Figure 1. Dendrogram resulting from cluster analysis of pair-wise genetic distances among 22 Puget Sound chinook population samples. Populations are fall-run unless otherwise indicated. R=River, H=hatchery, CR=Creek, SP=spring-run, SU=summer-run.

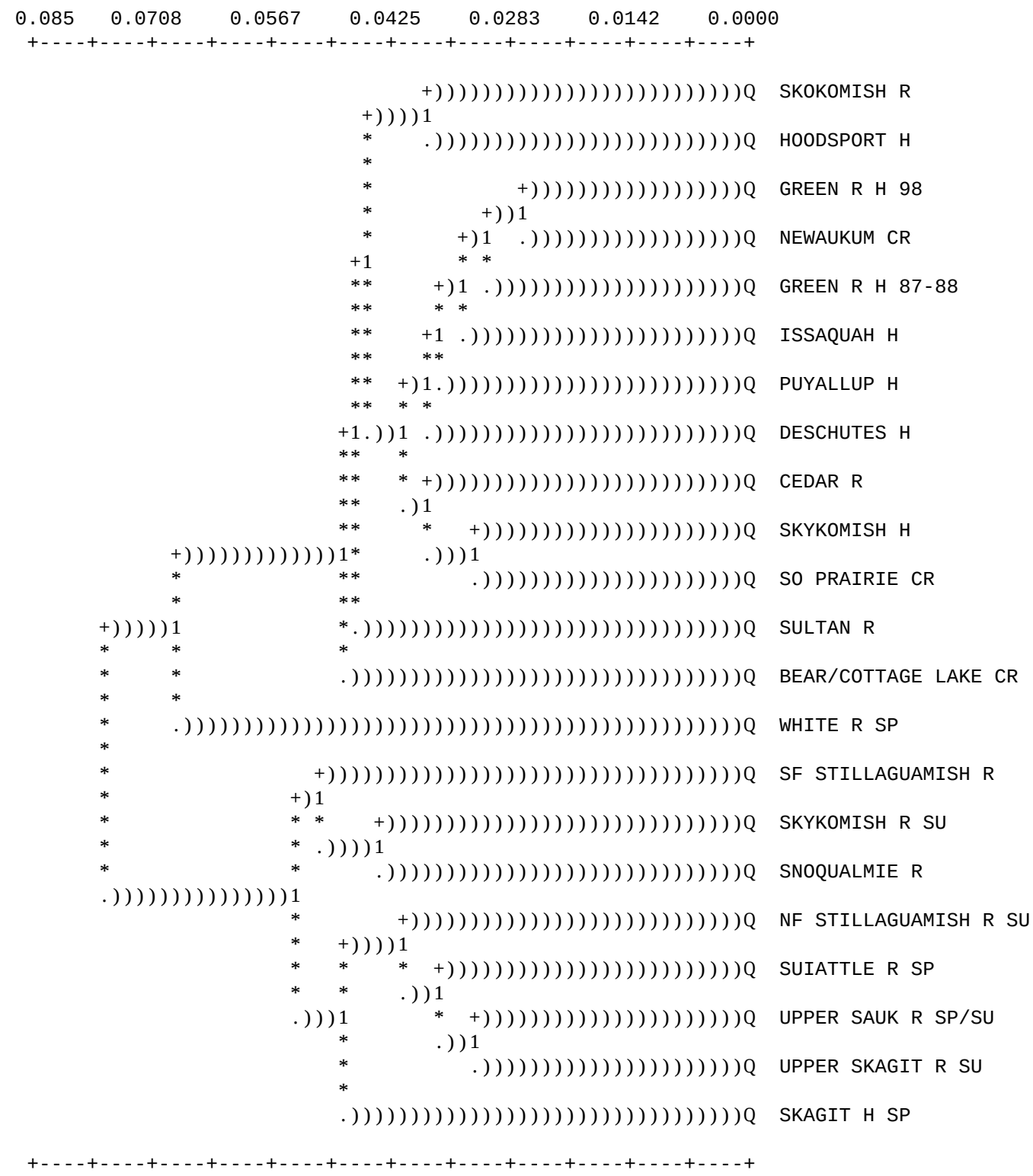

Figure 2. Three-dimensional scaling diagram of pair-wise genetic distances (Cavalli-Sforza and Edwards chord distance) among 15 southern Puget Sound chinook population samples. Populations are fall-run unless otherwise indicated. H=hatchery; see Table 1 for sample descriptions.